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Cloning and Characterization of the *Aeromonas caviae recA* Gene and Construction of an *A. caviae recA* Mutant

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A recombinant plasmid carrying the *recA* gene of *Aeromonas caviae* was isolated from an *A. caviae* genomic library by complementation of an *Escherichia coli recA* mutant. The plasmid restored resistance to both UV irradiation and to the DNA-damaging agent methyl methanesulfonate in the *E. coli recA* mutant strain. The cloned gene also restored recombination proficiency as measured by the formation of *lac*$^+$ recombinants from duplicated mutant *lacZ* genes and by the ability to propagate a strain of phage λ (*red gam*) that requires host recombination functions for growth. The approximate location of the *recA* gene on the cloned DNA fragment was determined by constructing deletions and by the insertion of Tn*5*, both of which abolished the ability of the recombinant plasmid to complement the *E. coli recA* strains. *A. caviae recA*::Tn*5* was introduced into *A. caviae* by P1 transduction. The resulting *A. caviae recA* mutant strain was considerably more sensitive to UV light than was its parent. Southern hybridization analysis indicated that the *A. caviae recA* gene has diverged from the *recA* genes from a variety of gram-negative bacteria, including *A. hydrophila* and *A. sobria*. Maxicell labeling experiments revealed that the RecA protein of *A. caviae* had an M_r of about 39,400.

The *recA* gene of *Escherichia coli* encodes a protein with a molecular weight of approximately 38,000 (2, 27). This protein is essential both to the SOS response and to homologous recombination (6, 29). The RecA protein is also responsible for the cleavage of temperate bacteriophage repressors, and thus is required for the induction of such phages as lambda, P22, and φ80 (12, 18, 29). Results of in vitro studies with purified RecA protein have demonstrated that it can perform several different enzymatic activities, including proteolysis of LexA and bacteriophage repressors as well as ATP hydrolysis, when it catalyzes the early steps of homologous recombination (14, 15, 17, 24, 29). Results of recent studies have suggested, however, that RecA may not be a protease, but may act indirectly as a positive effector of an autodigestion reaction performed by LexA and phage lambda repressors (16, 28).

There is ample evidence that RecA-like proteins with similar functions are present in a variety of gram-negative and gram-positive bacteria. Recently, the *recA* genes of several procaryotic species have been cloned and characterized with regard to their ability to complement *recA* mutants of *E. coli* (2, 7, 10, 11, 13, 21, 23). Results of these studies have demonstrated that the cloned *recA* genes are able to complement both the proteolytic functions and the recombination functions of the RecA protein. This occurs despite the finding that often there is little DNA sequence homology between *recA* genes from different species (13).

Aeromonas caviae is a gram-negative, oxidase-positive, facultatively anaerobic bacterium (25). Species of the genus *Aeromonas* are common inhabitants of aquatic environments. They have been implicated as pathogens of both warm- and cold-blooded animals (5). Recent attention has been focused on these microbes because of their increasing association with infection in humans (5). Additionally, Rippey and Cabelli (25) have shown that aeromonads can be used as trophic state indicators for aquatic environments.

We have recently begun to study the regulation of metabolism in *Aeromonas* species. The availability of a *recA* mutant of *A. caviae* would aid in its genetic analysis. In this report we describe the cloning and characterization of the *recA* gene from *A. caviae*. A Tn*5* insertion was introduced into the cloned *recA* gene, thereby inactivating it. This mutated gene was introduced into *A. caviae* by P1 transduction to construct a *recA* mutant strain.

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MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Tables 1 and 2, respectively.

Media. *E. coli* and *A. caviae* strains were routinely grown in Luria (L) broth or on L-agar plates (20). Antibiotics were used in the selection media at the following concentrations: ampicillin and kanamycin, 50 μg/ml; tetracycline, 20 μg/ml. Lac$^+$ papillae were detected on MacConkey lactose agar (Difco Laboratories, Detroit, Mich.). Methyl methanesulfonate (MMS; Sigma Chemical Co., St. Louis, Mo.) plates were prepared by spreading the appropriate amount of the MMS stock solution (11.6 M) on the surface of an L- or MacConkey agar plate containing 25 ml of agar and allowing it to dry for 4 to 6 h.

Isolation of *recA* clones. The *recA* clone of *A. caviae* was isolated by using a modification of the technique developed by Better and Helinski (2). *E. coli* HB101, a *recA* mutant, is incapable of growth on MMS, which is a mutagenic agent. *E. coli* HB101 clones which express a RecA$^+$ phenotype, and thus grow in the presence of MMS, were sought from a population of cells containing a plasmid gene bank constructed from *A. caviae* Y1 DNA.

The plasmid gene bank was constructed by using a directional cloning method (Fig. 1) (20). The plasmid vector pBR322 was digested to completion with the restriction enzymes *Bam*HI and *Hin*dIII, yielding two DNA fragments, a 0.35-kilobase (kb) fragment containing the tetracycline promoter and a 3.95-kb fragment containing the remainder of pBR322. The large 3.95-kb fragment was separated from the smaller fragment by agarose gel electrophoresis. The 3.95-kb fragment, which contained the ampicillin resistance gene, was eluted from the gel and ligated with *A. caviae* chromo-

TABLE 1. Bacterial strains and bacteriophages used in this study

Strain or bacteriophage	Relevent genotype or phenotype	Source or reference
E. coli		
HB101	F⁻ *hsdS20 recA13*	Maniatis et al. (20)
DR135	HB101 Ap^r *recA*$^+$ (pRecA35)	This study
DR303	HB101 Ap^r *recA*$^+$ (pDRA3)	This study
DR305	HB101 Ap^r Km^r *recA* (pDRA35)	This study
JC14604	F⁻ *lac* MS 2860811 *lacBK1* Δ(*srl-recA*) *hsr*	Clark and Marqulies (7)
SK8816	JC14604(pMK816)	Keener et al. (13)
SK1184	JC14604(pMK184)	Keener et al. (13)
SK7815	JC14604(pMK815)	Keener et al. (13)
SK7860	JC14604(pBR322)	Keener et al. (13)
DR505	JC14604(pDRA3)	This study
DR506	JC14604(pDRA35)	This study
DR507	JC14604(pRecA35)	This study
A. caviae		
Y1	*recA*$^+$	M. Rodgers
Y2	*recA*	This study
Phage		
Lambda::Tn*5*	*b*221 *rex*::Tn*5* c1857 *O*am6 *P*am29	Berg (1)
Lambda L47	*red gam*	Loenen and Brammar (19)

somal DNA which had been partially digested with the same two enzymes. Using this procedure, we found that >90% of the ampicillin-resistant (Ap^r) colonies contained recombinant plasmids. The plasmid gene bank was introduced into *E. coli* HB101 by calcium chloride transformation (20). Transformed *E. coli* HB101 colonies that expressed the RecA$^+$ phenotype were isolated by screening for resistance to both MMS (MMS^r) and UV light (UV^r) (1).

The *E. coli* ML-35 *recA* gene was cloned as described by Keener et al. (13). Briefly, chromosomal DNA from *E. coli* ML-35 was digested with *Bam*HI and ligated into the *Bam*HI site of pBR322. The ligated DNAs were transformed into *E. coli* HB101. Transformants that were Ap^r and MMS^r were purified and tested for resistance to UV radiation.

Recombinant DNA techniques. Plasmid DNA was isolated by the method described by Birnboim and Doly (3). For large-scale preparations, the plasmid DNA was purified by ethidium bromide-cesium chloride density gradient centrifugation (20). *E. coli* and *A. caviae* chromosomal DNAs were isolated by the technique described by Goldberg and Mekalanos (10). Restriction endonucleases were purchased from Bethesda Research Laboratories (Gaithersburg, Md.), and reaction conditions were as specified by the manufacturer. Restriction fragments were analyzed on 0.8 or 1.0% agarose gels in Tris borate EDTA (TBE) buffer (20).

Southern hybridizations. Genomic or plasmid DNA was digested to completion with the indicated restriction enzyme. Digested DNA was electrophoresed in 0.8% agarose gels with TBE buffer and transferred to nitrocellulose or nylon filters as described by Maniatis et al. (20). DNA probes were nick translated with [α-^{32}P]dCTP with a nick-translation kit (Bethesda Research Laboratories). Nitrocellulose filters were hybridized with a hybridization chamber (Hyrid-Ease PR800; Hoefer Scientific Instruments). The filters were prehybridized for 1 to 3 h at 65°C in 1.0 M NaCl–1% sodium dodecyl sulfate (SDS). The radioactive

TABLE 2. Plasmids used in this study

Plasmid	Insert DNA	Vector	Source or reference
pBR322	ColE1 Ap^r Tc^r		Maniatis et al. (20)
pRecA35	*E. coli* ML35 *recA* region	pPR322	This study
pDRA3	*A. caviae recA* region	pBR322	This study
pDRA35	pDRA3 *recA*::Tn*5*	pBR322	This study
pMK710	*Erwinia carotovora recA* region	pBR322	Keener et al. (13)
pMK816	*S. flexneri recA* region	pBR322	Keener et al. (13)
pMK184	*E. coli* B/r *recA* region	Yrp12	Keener et al. (13)
pMK815	*P. vulgaris recA* region	pBR322	Keener et al. (13)
pBR322-*recA*$^+$	*E. coli* K-12 *recA* region	pBR322	Keener et al. (13)

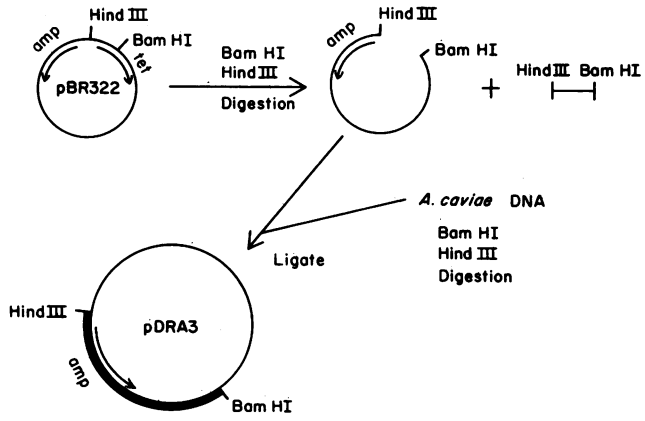

FIG. 1. Construction of an *A. caviae* plasmid gene bank by directional cloning. First, pBR322 was digested with *Bam*HI and *Hin*dIII. The two restriction fragments were then separated by agarose gel electrophoresis. Finally, the larger fragment, which contained the ampicillin resistance gene, was ligated with *A. caviae* chromosomal DNA which had been restricted with the same enzymes.

probe was denatured by boiling for 5 min and then rapidly cooled in an ice water bath. The denatured probe was mixed with the hybridization solution (1.0 M NaCl, 1% SDS, and 100 μg of denatured salmon sperm DNA per ml). Hybridization was done at 65°C for 14 to 18 h. The filters were then given two low-stringency washes (2× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 1% SDS) of 5 and 30 min, followed by two high-stringency washes (0.1× SSC, 1% SDS; 30 min each). Autoradiography was performed for 5 to 48 h at −70°C with X-Omat AR film (Eastman Kodak Co., Rochester, N.Y.) and an intensifying screen (Du Pont Co., Wilmington, Del.).

UV and MMS survival measurements. Cells were grown in L broth supplemented with the appropriate antibiotics to a density of 1×10^8 to 5×10^8 cells per ml at 37°C for *E. coli* and 30°C for *A. caviae*. The cells were collected by centrifugation, washed in phosphate-buffered saline (pH 7), and suspended to 10^8 cells per ml in the same buffer. Ten-milliliter samples of washed cells were placed in the bottoms of plastic petri dishes (100 by 15 mm) and then irradiated with a 254-nm UV lamp (model UVS-54; Ultra-violet Products, Inc., San Gabriel, Calif.). After UV irradiation, the samples were placed in ice, serially diluted, and spread onto L agar. After 16 to 20 h of incubation at 35°C, cell survival was determined by counting the number of CFU per plate.

Sensitivity to MMS was determined by using cells prepared as described above. Washed cells were serially diluted and spread directly onto L agar containing the appropriate concentration of MMS. Cell survival was determined as described above.

Measurement of recombination proficiency. Recombination proficiency was determined with *E. coli* JC14604 (13). This strain contains a duplication of the *lacZ* region and is a *recA* mutant. Each copy of the *lacZ* gene contains a different missense mutation and therefore expresses a $LacZ^-$ phenotype. Introduction of a plasmid containing a complementing *recA* gene into JC14604 allowed homologous recombination to occur. This resulted in the appearance of Lac^+ papillae after 48 h on lactose MacConkey agar (13). By comparing the number of Lac^+ papillae produced by the heterologous *recA* genes to that produced by a cloned *E. coli recA* gene, a quantitative measure of recombination proficiency could be determined.

E. coli JC14604 was transformed with the indicated plasmids by the calcium chloride method (20). The appropriate volume of transformation mixture to yield 50 to 100 CFU per plate was spread onto the surface of a MacConkey lactose agar plate. The Lac^+ papillae were scored after 48 h at 37°C.

Recombination proficiency was also determined by measuring the efficiency of plating of lambda L47 *red gam* on *recA* plasmid-containing strains. Lambda L47 is deficient in phage-mediated recombination, and thus can only form mature phages with a $RecA^+$ host strain. Host cells were grown overnight in L broth containing 0.2% maltose. The culture was concentrated by centrifugation and suspended to half the original volume in 20 mM Mg_2SO_4. Concentrated cells (200 μl) were infected with the appropriate phage dilution and incubated at 37°C for 15 min. The cells were added to 0.7% L soft agar and plated onto L agar. After 16 to 20 h of incubation at 37°C, recombination proficiency was determined by counting the number of PFU.

Induced recombination assay. Induced recombination assays were performed in the same manner as described above for the JC14604 recombination proficiency assay. However, fractions of JC14604 transformed with $recA^+$ plasmids were plated onto MacConkey agar plus MMS or exposed to UV light after they were plated on MacConkey agar. Transformants plated on MacConkey agar alone served as a control. Lac^+ papillae were scored after 48 h at 37°C.

Transposon mutagenesis of the cloned *A. caviae recA* gene. The procedure described by Downard et al. (8) was used to obtain pDRA3 containing Tn*5* inserts. *E. coli* DR303 was grown to 60 Klett units (1.0×10^9 cells per ml) in L broth containing 0.2% maltose. The culture was infected with lambda::Tn*5* at a multiplicity of infection of 1. The infected culture was incubated at 37°C for 15 min and then concentrated 25-fold before it was spread onto L agar containing kanamycin. Plasmid DNA was then isolated from kanamycin-resistant (Km^r) *E. coli* and used to transform *E. coli* HB101. The resulting Km^r colonies of HB101 were screened for Tn*5* inserts into the cloned *recA* gene by replica plating onto MMS and screening for the loss of the $RecA^+$ phenotype. Confirmation of the Tn*5* insert into the cloned *recA* gene was determined by restriction mapping.

Construction of an *A. caviae recA* mutant. *recA* mutants of *A. caviae* were selected from a population of *A. caviae* Y1 cells transduced with pDRA35 (the *recA*::Tn*5* insertion mutant of pDRA3). Briefly, *E. coli* C600 was transformed with pDRA35 (19). Phage P1 was then grown on the transformants, and the lysate was used to transduce *A. caviae* Y1 (22). Km^r colonies were picked and tested for UV^s. Clones that were Km^r and UV^s were considered to be *recA* mutant strains. Since both P1 and ColE1 replicons are unable to replicate in *A. caviae* (unpublished data), it was considered that Km^r clones resulting from the transduction with the P1 lysate of C600(pDRA35) represented either (i) Tn*5* transpositions into *A. caviae* or (ii) a crossover event between *recA* and *recA*::Tn*5* resulting in the substitution of *recA*::Tn*5* for *recA*. These two possibilities were differentiated by screening for UV^s. All Km^r clones tested were UV^s, indicating that crossover events were much more common than Tn*5* hops.

Maxicell labeling and analysis of *recA* gene products. Maxicell labeling experiments were carried out by a modification of the technique of Sancar et al. (26), as described by Blackhart and Zusman (4). Briefly, *E. coli* JC14604 or *E. coli* JC14604 transformed with pDRA3 or pDRA35 was grown at 34°C to 5×10^8 cells per ml. Fractions of 5 ml were pipetted into petri plates and exposed to 200 J of UV light per m^2. The irradiated cells were placed in aluminum foil-wrapped tubes and incubated at 34°C for 2 h. Cycloserine (200 μg/ml) was added, and the incubation was continued for 16 h. The cells were centrifuged (5,000 × *g* for 10 min), washed once with 5 ml of sulfate-free minimal medium, and suspended in 5 ml of sulfate-free minimal medium containing 200 μg of cycloserine per ml. The cells were incubated at 34°C for 1 h and then labeled with 10 μCi of [^{35}S]methionine for 2 h. The labeled cells were collected by centrifugation, suspended in 150 μl of protein solubilization buffer (15), and boiled. Samples of 30 μl were then analyzed by electrophoresis on 12% SDS-polyacrylamide gels (15) followed by fluorography.

RESULTS

Isolation of the *A. caviae recA* gene. A recombinant plasmid containing the *A. caviae recA* gene was isolated from a gene library constructed in pBR322 and propagated in the *recA* mutant *E. coli* HB101. The library was screened for colonies resistant to the DNA-damaging agent MMS or to UV irradiation (2, 13). Only colonies in which the *E. coli recA* mutation was complemented by an *A. caviae recA* gene were able to grow on these plates after an overnight incubation at 37°C. Of approximately 1,000 ampicillin-resistant colonies

screened, 5 were found to contain plasmids with *A. caviae* DNA that was capable of complementing the *recA* mutation in HB101. Plasmid DNA was isolated from the five MMS^r colonies and used to transform *E. coli* HB101. Plasmids from all five colonies again conferred MMS^r. Agarose gel electrophoresis revealed that three of the five plasmids contained recombinant DNA of the same size (data not shown). The largest of the clones isolated, pDRA1, contained a 19-kb insert of *A. caviae* DNA. Plasmids pDRA2, pDRA3, and pDRA4 each contained approximately 5 kb of *A. caviae* DNA, while pDRA5, the smallest clone, contained a 3-kb insert.

Restriction and deletion mapping of the *recA* gene. Restriction endonuclease mapping of three plasmids that encoded MMS^r (pDRA1, pDRA3, pDRA5) revealed that all three plasmids contained two common *Pst*I fragments of 1.1 and 0.9 kb. Additionally, pDRA3 and pDRA5 also shared a common 1.5-kb *Pst*I fragment. Digestion with *Bgl*II showed that the three plasmids all contained a common 0.6-kb fragment. Since the three MMS^r plasmids shared two common *Pst*I fragments, it was considered possible that *A. caviae recA* was located on either one or both of these fragments. To test this prediction, pDRA3 was completely digested with *Pst*I, the fragments were ligated, and the resulting plasmids were used to transform *E. coli* HB101. Transformants were plated in L agar containing MMS. Only those cells that received a functional *recA* gene could grow. Several hundred MMS^r colonies were obtained. Restriction analysis performed on plasmids extracted from 10 random MMS^r clones revealed that each had both the 0.9- and 1.1-kb *Pst*I fragments. No MMS^r clones were found that contained only one of the common *Pst*I fragments. These data suggest that sections of the *recA* gene are located on both the 0.9- and 1.1-kb *Pst*I fragments.

Since all three original MMS^r plasmids contained a common 0.6-kb *Bgl*II fragment, it was thought likely that the *recA* gene was located on that fragment. A derivative of pDRA3 from which the 0.6-kb *Bgl*II fragment was deleted was constructed and used to transform HB101. Transformants were screened for MMS^r or MMS^s. All clones from which the 0.6-kb *Bgl*II fragment was deleted from pDRA3 were MMS^s, indicating that this fragment contains sequences that are essential for the function of *recA*. The data presented above, combined with additional restriction analysis, were used to construct a restriction map of the 5-kb insert of pDRA3 (Fig. 2).

Transposon mutagenesis and mapping. The transposon Tn*5* was used to mutagenize and locate the *A. caviae recA* gene within the 5-kb insert of pDRA3. Tn*5* was introduced into *E. coli* DR303(pDRA3) by using lambda::Tn*5* (8). *E. coli* HB101 cells harboring pDRA3::Tn*5* were then tested for MMS^r. If Tn*5* had inserted into the *recA* gene, it would have inactivated that gene and the clone would have reverted to the MMS^s phenotype. Of the 25 Km^r colonies tested, one contained a plasmid which no longer conferred MMS^r. This Tn*5*-containing plasmid was designated pDRA35. Restriction mapping of pDRA35 revealed that the transposon inserted into the 1.1-kb *Pst*I fragment (Fig. 2).

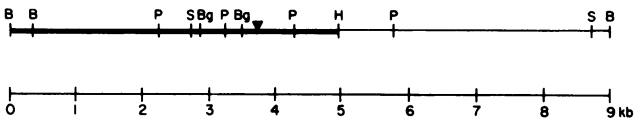

FIG. 2. Restriction map of pDRA3. The heavy line represents the cloned *A. caviae recA* region, and the light line represents the pBR322 sequences. Restriction enzyme sites are abbreviated as follows: B, *Bam*HI; Bg, *Bgl*II; H, *Hin*dIII; P, *Pst*I, S, *Sal*I. Restriction sites for *Cla*I and *Eco*RI were not found in the *recA* region of pDRA3. The inverted triangle indicates the location of the Tn*5* insert which inactivates the RecA functions.

Complementation studies with the cloned *A. caviae recA* gene. The ability of the cloned *A. caviae recA* gene in pDRA3 to complement both a defective SOS response and defective recombination functions was determined. Quantitative UV and MMS survival experiments compared the ability of wild-type *A. caviae* Y1, *E. coli* HB101, *E. coli* HB101 harboring either pDRA3 or pRecA35, *E. coli* HB101 with pDRA35 (the Tn*5* insertion mutant), and *A. caviae* Y2 (transduced to *recA* with pDRA35) to survive exposure to UV irradiation or MMS. The results of these experiments are shown in Fig. 3. In both the UV and MMS survival experiments, the *A. caviae recA* clone (pDRA3) was able to complement the *recA* mutant HB101 close to the levels exhibited by the *E. coli recA* clone (pRecA35). This was surprising since *A. caviae* Y1 was found to be somewhat sensitive to both DNA-damaging agents. For example, UV irradiation of *A. caviae* Y1 at 9 J/m^2 caused a 4 log reduction in CFU. However, when HB101 was transformed with either pDRA3 or pRecA35 and then irradiated, only about a 1 log reduction in CFU was observed (Fig. 3B). In contrast, strain HB101 transformed with pDRA35 or *A. caviae* transduced with pDRA35 (*A. caviae* Y2) was extremely UV^s and considered to be a *recA* mutant.

The ability of pDRA3 to promote homologous recombination was determined by two methods. First, the plaque-forming efficiency of a *red gam* deletion mutant lambda phage (lambda L47) that is unable to grow in a *recA* mutant host strain was measured. The plaque-forming efficiency of lambda L47 on HB101 was 0.01 relative to that of *recA*$^+$ *E. coli* WL66 (Table 3). Transformation of pDRA3 into HB101 was able to restore the plating efficiency of lambda L47 to 92% of that observed in WL66. Similarly, pRecA35 transformed into HB101 allowed lambda L47 to form plaques at levels equal to or higher than those observed for WL66. Furthermore, pDRA35, the putative Tn*5* insertion into the *recA* region of pDRA3, did not restore plaque-forming efficiency when transformed into HB101.

Recombination proficiency was also determined with *E. coli* JC14604 (see above). Transformants containing pDRA3 had a recombination proficiency of approximately 40% relative to that of cells containing pRecA35 (Table 4). This assay was also used to determine the recombination proficiency of several other cloned *recA* genes (Table 4). pMK816 (*Shigella flexneri*), pMK815 (*Proteus vulgaris*), and pMK710 (*Erwinia carotovora*) each had a recombination proficiency of approximately 50%. Interestingly, pMK184 (*E. coli* B/r) had a recombination proficiency of only 33%. This was the lowest rate observed among the *recA* plasmids tested. Neither pBR322 nor pDRA35 (*A. caviae recA*::Tn*5*) promoted recombination.

Induced recombination. Since the expression of *recA* is induced by DNA damage, we were interested in whether DNA-damaging agents would cause an increased rate of homologous recombination. The recombination assay with strain JC14604 was used to measure DNA damage-induced recombination. JC14604 cells transformed with *recA*-bearing plasmids were plated on MMS or plated and irradiated with UV light. The Lac$^+$ papillae were scored after 48 h. The increase in recombination proficiency was calculated by comparing the number of Lac$^+$ papillae per CFU on control plates not exposed to DNA-damaging agents with the num-

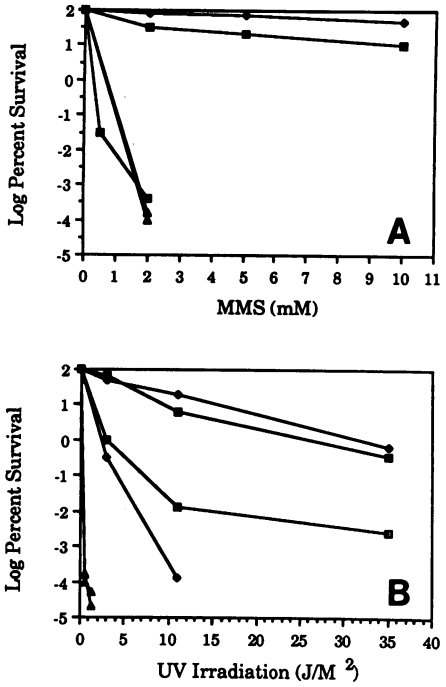

FIG. 3. Survival of *recA*$^+$ and *recA* cells on exposure to MMS and UV light. *E. coli* HB101 (△), DR303 (■), DR135 (◇), and DR305 (▲); *A. caviae* Y1 (□); and *A. caviae* Y2 (◆) were exposed to increasing amounts of either MMS (A) or UV light (B); and the percentage of surviving cells was determined by comparison with the same strains not exposed to either DNA-damaging agent.

ber of papillae per CFU on plates containing the DNA-damaging agent. We observed a 5.0-fold increase in recombination when DR505 (*A. caviae recA*) was subjected to UV irradiation (Table 5). A 4.3-fold increase was observed in DR507 (*E. coli recA*). When the cells were exposed to 2.5 mM MMS, DR505 experienced a 15.3-fold increase in recombination, while DR507 had a 2.5-fold increase. Cells transformed with either pBR322 or pDRA35 exhibited no recombination, even when exposed to MMS or UV.

The relationship between the amount of DNA-damaging agent (and therefore the amount of DNA damage) and recombination was also investigated. *E. coli* DR505 (JC14604 harboring pDRA3) was plated onto MacConkey agar plates containing 0 to 6 mM MMS. After 48 h at 37°C, the Lac$^+$ papillae were scored. The results of this experiment (Fig. 4) indicate that as the amount of the DNA-damaging agent (MMS) increased, the amount of recombination increased proportionately. The same experiment was also attempted with *E. coli* DR507(pRecA35). However, this

TABLE 3. Efficiency of plating lambda L47 on *recA* plasmid-containing strains

Host strain	Plating efficiency[a]
WL66	100
HB101	0.01
DR506(pRecA35)	106
DR503(pDRA3)	91.7
DR505(pDRA35)	0.01

[a] Relative to the plating efficiency on RecA$^+$ *E. coli* WL66. Phage from a stock of approximately 2 × 10^8 PFU/ml were plated onto each of the host strains. Cell plating and preparation was as described in the text.

TABLE 4. Recombination proficiency of JC14604 harboring *recA* clones

Plasmid	% Recombination[a]
pRecA35	100
pMK816	52
pMK815	51
pMK710	52
pMK184	33
pDRA3	42
pDRA35	0.0
pBR322	0.0

[a] The indicated plasmids were introduced into JC14604 by calcium chloride transformation and plated onto MacConkey lactose agar. Lac$^+$ papillae were scored after 48 h at 37°C.

strain had a recombination proficiency that was almost 60% greater than that of DR505(pDRA3) (Table 4). Therefore, an increase in DNA damage resulted in an uncountable number of Lac$^+$ papillae per CFU.

Sequence homology. By use of pDRA3 (*A. caviae recA* region) as a hybridization probe, the sequence homologies among the *recA* genes of *A. caviae*, *E. coli*, and several other gram-negative bacteria were investigated (Fig. 5B). Among the strains tested only slight homology with the *A. caviae recA* region could be detected in *E. coli* ML-35(pRecA35), *E. coli* B/r(pMK184), *E. coli* K-12(pBR322-*recA*$^+$), *S. flexneri* (pMK816), or *Erwinia carotovora* (pMK710). No homology could be detected between *P. vulgaris* (pMK815) and the *A. caviae recA* gene regions.

The Southern blot was then rehybridized with pRecA35 (*E. coli* ML-35 *recA* region) as a probe (Fig. 5A). The results were similar to those obtained by Keener et al. (13) when the *E. coli* K-12 *recA* gene was used as a probe. *E. coli* ML-35(pRecA35), *E. coli* B/r(pMK184), *E. coli* K-12(pBR322-*recA*$^+$), and *S. flexneri* (pMK816) showed strong homology. Slight homology could be detected with *Erwinia carotovora* (pMK710) and *A. caviae* (pDRA3). No homology could be detected with *P. vulgaris* (pMK815).

pDRA3 (*A. caviae recA* region) was also used to probe total chromosomal DNA from other *Aeromonas* species (Fig. 6). Homology was detected between *A. caviae* and two related species, *A. hydrophila* and *A. sobria*. The *recA* genes of all three strains were contained in different-sized *Bam*HI fragments. The *recA* gene of *A. caviae* was in a 6.0-kb fragment, while that of *A. hydrophila* is present in a 4.4-kb *Bam*HI fragment; and the *A. sobria recA* gene was in a 9.0-kb *Bam*HI fragment.

Determination of RecA protein molecular weight. The approximate molecular weight of the *A. caviae* RecA protein

TABLE 5. Induced recombination

Strain	Fold recombination increase after damage by[a]:	
	UV light	MMS
DR505(pDRA3)	5.9	15.3
DR507(pRecA35)	4.3	2.5
SK7860(pBR322)	0.0	0.0
DR506(pDRA35)	0.0	0.0

[a] *E. coli* JC14604 harboring the indicated plasmids were plated onto MacConkey lactose agar. The cells were then exposed to the DNA-damaging agents of UV light (3 J/m^2) or MMS (2.5 mM) as described in the text. A second group of plates was prepared which were not exposed to the DNA-damaging agents. The increase in recombination proficiency was calculated by comparing the number of Lac$^+$ papillae per CFU.

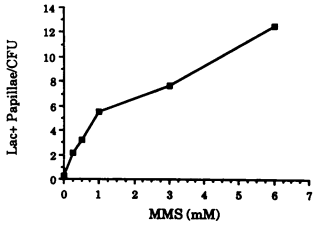

FIG. 4. The effect of MMS on the rate of recombination. *E. coli* DR505 cells were plated onto MacConkey agar containing the indicated concentrations of MMS. Lac^+ papillae were scored after 48 h at 37°C.

was determined by maxicell labeling (4, 26) and analysis by SDS-polyacrylamide gel electrophoresis and fluorography (Fig. 7). The M_r of the *E. coli* RecA protein was estimated to be 37,500, which was in good agreement with the actual molecular weight of 37,842 reported by Sancar et al. (27). The *A. caviae* RecA protein was determined to have an M_r of 39,400, which is about 4% larger than the *E. coli* RecA protein.

DISCUSSION

The RecA protein acts to derepress the SOS response and to promote homologous recombination (13, 29). Thus, cells that are *recA* mutants are extremely sensitive to DNA damage and are unable to perform recombination functions (6, 24, 29). We cloned and partially characterized the *recA* gene of *A. caviae*. The 5-kb *Bam*HI-*Hin*dIII fragment of *A. caviae* DNA that contains the *recA* gene complements a wide range of *recA* phenotypes, including MMS and UV sensitivity, inability to propagate *red gam* mutant strains of lambda phage, and the lack of homologous recombination. Furthermore, we were able to locate the *recA* gene within the cloned 5-kb fragment by constructing both a *Bgl*II deletion and a Tn*5* insertion within the cloned gene (Fig. 2). Both the deletion and the insertion prevented the cloned DNA fragment from complementing *recA* mutant strains of *E. coli*. Additionally, a *recA* mutant strain of *A. caviae* was constructed by replacing the wild-type *recA* gene with one that contained a Tn*5* insertion.

It has been hypothesized that the RecA protein and possibly other components of the SOS regulon have been functionally conserved through evolution (13). That the *A. caviae recA* gene, when cloned into HB101, restores RecA functions is consistent with this hypothesis. Southern hybridization experiments indicated that only slight DNA sequence homology exists between the *recA* gene of *A. caviae* and *E. coli* K-12, B/r, and ML-35; *S. flexneri*; and *Erwinia carotovora*. Under conditions of stringent hybridization, no homology was detectable between *A. caviae* and *P. vulgaris recA* regions. Our data are in agreement with those of Keener et al. (13). They reported that when the cloned *E. coli* K-12 *recA* gene is used as a probe, there is sufficient DNA sequence homology to detect hybridization only to *S. flexneri* and *E. coli* B/r. No hybridization was seen to *P. vulgaris* and *Erwinia carotovora recA* genes. These data, taken together with the conserved cross-reactivity of the various RecA proteins also reported by Keener et al. (13), strongly suggest that function as well as specific epitopes have been evolutionarily conserved, even though changes in the DNA base sequence that abolish cross-hybridization under moderate to stringent conditions have occurred.

When chromosomal DNAs of three *Aeromonas* species were digested with *Bam*HI and probed with the *A. caviae recA* gene, homology was detected among *A. caviae*, *A. hydrophila*, and *A. sobria*. However, the *recA* gene of each species was contained in different-sized *Bam*HI fragments (Fig. 6). Until recently, *A. caviae* and *A. sobria* were considered to be strains of *A. hydrophila* (5). Our results are consistent with the reclassification of these organisms. This is supported by the finding that in the three *E. coli* strains investigated (K-12, B/r, and ML-35), the *recA* gene was contained within identically sized *Bam*HI fragments (Fig. 5).

Interestingly, while we were able to clone the *A. caviae*

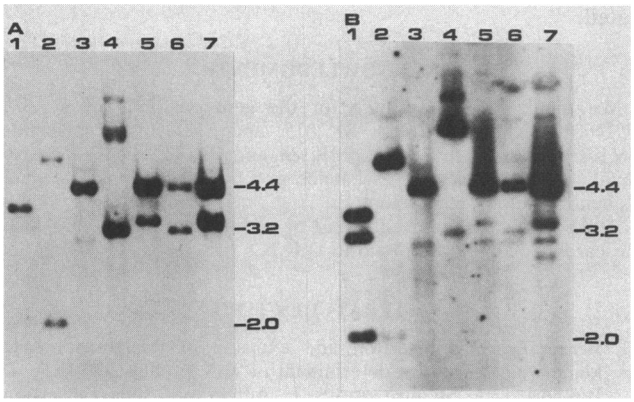

FIG. 5. Hybridization of the *E. coli* and *A. caviae recA* genes to the cloned *recA* regions from heterologous bacterial strains. Plasmid DNA containing the *recA* region from various bacteria was digested with the indicated restriction endonucleases and electrophoresed in a 0.8% agarose gel. The DNA fragments were transferred to a nitrocellulose filter and hybridized to either ^{32}P-labeled, nick-translated pRecA35 (A) or to ^{32}P-labeled, nick-translated pDRA3 (B). The size of the restriction fragment containing the *recA* region and the restriction enzyme(s) used are given below: lanes 1, pDRA3 (*Hin*dIII-*Sal*I; 2.0 kb); lanes 2, pRecA35 (*Bam*HI-*Eco*RI; 2.0 kb); lanes 3, pMK710 (*Bam*HI; 5.2 kb); lanes 4, pMK184 (*Bam*HI; 3.2 kb); lanes 5, pMK815 (*Bam*HI; 14.8 kb); lanes 6, pBR322-*recA*$^+$ (*Bam*HI; 3.2 kb); lanes 7, pMK816 (*Bam*HI; 3.3 kb).

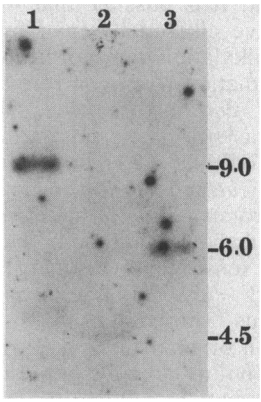

FIG. 6. Southern hybridization analysis of the *recA* regions of *Aeromonas* species. Chromosomal DNA from *A. sobria* HSI (2 μg: lane 1); *A. hydrophila* M8 (2 μg; lane 2); and *A. caviae* Y1 (1 μg; lane 3) was digested to completion with *Bam*HI and electrophoresed in a 0.7% agarose gel. The DNA fragments were transferred to a nitrocellulose filter and hybridized to ^{32}P-labeled, nick-translated pDRA3. The approximate size of each fragment is given to the right of the gel, in kilobases.

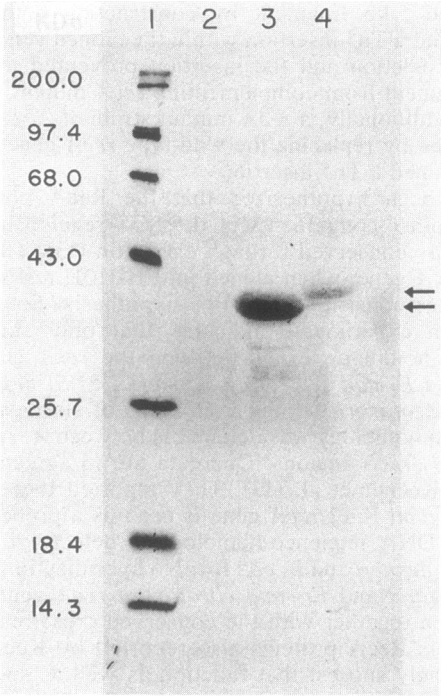

FIG. 7. Maxicell labeling experiment for the determination of the molecular weight of cloned RecA proteins. Plasmid-encoded proteins were labeled by using the modification of the method of Sancar et al. (26), as described by Blackhart and Zusman (4). *E. coli* JC14604 (lane 2) was transformed with pRecA35 (lane 3) or pDRA3 (lane 4). Molecular weight standards (lane 1) are given to the left of the gel, in thousands. The positions of the RecA proteins are indicated by arrows.

recA gene by complementation of HB101 to MMS^r and UV^r, the parent wild-type strain of *A. caviae* was relatively sensitive to MMS and UV irradiation (Fig. 3). The data presented here demonstrate that the cloned *A. caviae* gene is induced by DNA damage and is capable of derepressing the SOS response. What, then, could be the reason for the sensitivity of *A. caviae* Y1 to MMS and UV? First, MMS is an alkylating agent. It is possible that *A. caviae* does not possess an effective alkylation repair system and cannot effectively repair alkylation damage. Second, while Y1 was sensitive to UV radiation when compared with the sensitivity of wild-type *E. coli* and *E. coli* HB101 complemented with pDRA3, it was still much more resistant to UV light than was either HB101 or Y2 (Fig. 3). Furthermore, when the ability of *A. caviae* and *Vibrio cholerae* to withstand UV irradiation (10) is compared with that of *recA*$^+$ strains of *E. coli*, it can be seen that both aquatic bacterial species are considerably more sensitive to UV light. However, both the *A. caviae* and the *V. cholerae recA* genes fully complement *E. coli recA* mutants. These data suggest that some portion of the SOS regulon other than *recA*, at least in these two aquatic species, is not as effective in protecting or repairing damage by UV irradiation as it is in *E. coli*.

The ability of the cloned *A. caviae* RecA protein to promote recombination appeared similar to that of the *E. coli* RecA protein. The growth of *red gam* mutants of lambda were restored to wild-type levels in strain HB101 complemented with pDRA3 (Table 3), and recombination between *lacZ* missense mutants to produce *lacZ*$^+$ cells was similarly complemented by the introduction of pDRA3 into strain JC14604 (Table 4). As noted above, recombination functions of the RecA protein appeared to be highly conserved, even though the DNA sequence homology was not. Thus, the *E. coli recA* gene cloned from strain ML-35(pRecA35) and the *recA* gene from B/r(pMK184), which exhibit the highest levels of homology, yielded the highest and lowest rates of recombination, respectively (Table 4). In contrast, *recA* genes from *A. caviae*, *P. vulgaris*, *Erwinia carotovora*, and *S. flexneri*, which have various amounts of sequence homology with each other and with *E. coli recA*, all complemented recombination to similar levels (Table 4). It is likely that quantitative differences between the ability of RecA proteins to catalyze recombination functions in *E. coli* may reflect differences in the expression in *E. coli* of *recA* genes from different genera.

Induced recombination assays demonstrated that when *E. coli* JC14604 harboring a *recA*-containing plasmid was exposed to the DNA-damaging agents MMS and UV, there was a dramatic increase in recombination proficiency (Table 5). The increase in recombination was proportional to the amount of DNA-damaging agent to which the cells were exposed (Fig. 4). There are several possible explanations for this observation. (i) The DNA-damaging agents could have activated the SOS response, and thus increased the amount of cellular RecA protein and the amount of recombination; or (ii) the DNA-damaging agents could have generated increased amounts of single-stranded DNA, which is one of the requirements for the RecA protein in homologous recombination. It is also possible that the increase in homologous recombination was due to a combination of the two possibilities mentioned above. In other words, DNA-damaging agents increase the amount of single-stranded DNA, which activates the SOS response, causing an increase in RecA protein. Since there is more single-stranded DNA and more RecA protein, there is more homologous recombination. It should also be pointed out that the increase in recombination frequency in JC14604 transformed with pDRA3 suggests, but does not prove, that the *A. caviae recA* gene may be under the control of *E. coli lexA*. This possibility will be investigated.

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